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SUCCESSION IN A MICROBIAL COMMUNITY ASSOCIATED WITH CHITIN IN LAKE ERIE SEDIMENT AND WATER[1]

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Abstract. Slides coated with reprecipitated chitin were buried in sediments from Lake Erie under laboratory conditions. Changes in the microbial community associated with the slide were noted over time, in depth of the water and sediment column, and in anaerobic and aerobic zones of the sediment. Bacterial activity in the overlying water was greatest after 2 to 7 days of incubation whereas sediment populations showed greatest numbers from 7 days (aerobic zone) to 13 days (anaerobic zone). Control slides showed no change in the time period used. Rod to ovoid forms predominated in the water regime and eventually (20 days) showed no difference from the control slides. Rod-shaped forms dominated sediment population up to approximately 10 days at which time vibrioid and/or spiral forms became the dominant flora until complete hydrolysis was evident.

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Microbial populations active in decomposition of chitin, a major macromolecule in aquatic and terrestrial habitats, have been studied by a number of techniques. Often isolates from an environment are taken and tested for activity in the laboratory (Campbell and Williams 1951, Clarke and Tracey 1956). Others have devised methods of enumerating chitinolytic organisms by most probable number (MPN) (Skinner and Dravis 1937) or plate count methods (Zobell and Rittenberg 1938). All of these methods yield information about the specific isolate more than about their ecological significance. The importance of sediments and other particulates in mixed populations cannot be discounted in light of findings by Weaver and Dugan (1972) and Hargrave and Phillips (1977) indicating the stimulatory effect they usually have on microbial activity. This project was designed to determine changes over time in the microbial population of Lake Erie sediment and water associated with chitinous substrates in the environment. The contact slide method allows for examination of bacterial population in sediments, soils and waters and has been used by Gray and Bell (1963) and Okofar (1966) in studying chitin decomposition in water and soils, respectively. In the present study, a modified contact slide technique of Tribe (1957) has been used to study the population of microorganisms associated with a chitin coated buried slide in sediment and water of Lake Erie.

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MATERIALS AND METHODS

Sediment and water collection and storage. Sediment was collected approximately 30 m north of Rattlesnake Island in the Bass Island Region of Lake Erie during October 1973. An Ekman dredge was used for taking the sample in approximately 6.1 m water depth. The water overlay (approximately 2 cm) and the sediment were allowed to equilibriate for a month at room temperature (22 °C) to simulate *in situ* conditions in a 4 ℓ beaker.

Buried slide technique. The technique used has been previously described by Warnes and Randles (1977). Acid and alcohol cleaned slides were coated with reprecipitated chitin and fixed to the slide by heating. Slides were placed in the jar to allow for penetration of the anaerobic (>2 cm) and aerobic (<2 cm) zones of the sediment as well as for the air-water interface. Examination of varying regimes therefore could be examined on a single slide. Slides were removed after 2, 4, 6, 7, 10, 13, and 20 days incubation in the mud jar. They were washed to remove dirt, stained with safranin, examined and photographed with a Zeiss 16010 Micrscope at a magnification value

of 400 ×. Uncoated control slides were removed after 2 and 7 days incubation and treated in a similar manner. Observations of degree of hydrolysis, relative numbers of organisms adhering to the slides and morphologic types were recorded by photography.

RESULTS

Table 1 indicates the changes occurring to slides over a 20 day incubation period in the water and sediment zones. Variations in degree of hydrolysis (associated with clearing on the surface of the slides), bacterial numbers and different morphologic types are noted. The most rapid hydrolysis and colonization is seen at the air-water interface. After 6 days, the film of chitin had nearly disappeared, followed by a drop in bacterial numbers and morphologic types. Rods of approximately 3 μm in length predominated until the film disappeared. Figure 1a through d shows morphologic types seen at the air-water interface at various time periods. The rod-shaped organisms seen in 1c were the predominant type. Figure 1a shows cells adhering to the chitin coated slide after a 4 day incubation period, but little hydrolysis is evident. Large granulated rods seen at the water-air interface at 6 days (fig. 1b) are beginning to show hydrolysis on the chitin coated surface. By seven days, most of the chitin film is gone with rods (fig. 1c) and ovoid cells (fig. 1d) predominating.

Growth was also rapid in the water column itself. Figure 2a shows some morphologic forms observed at 6 days. Little diversity was seen in this zone. Rods and ovoid cells predominated.

The water-sediment interface and aerobic sediment (2 cm) showed a variety of morphologic types over the 20 day incu-

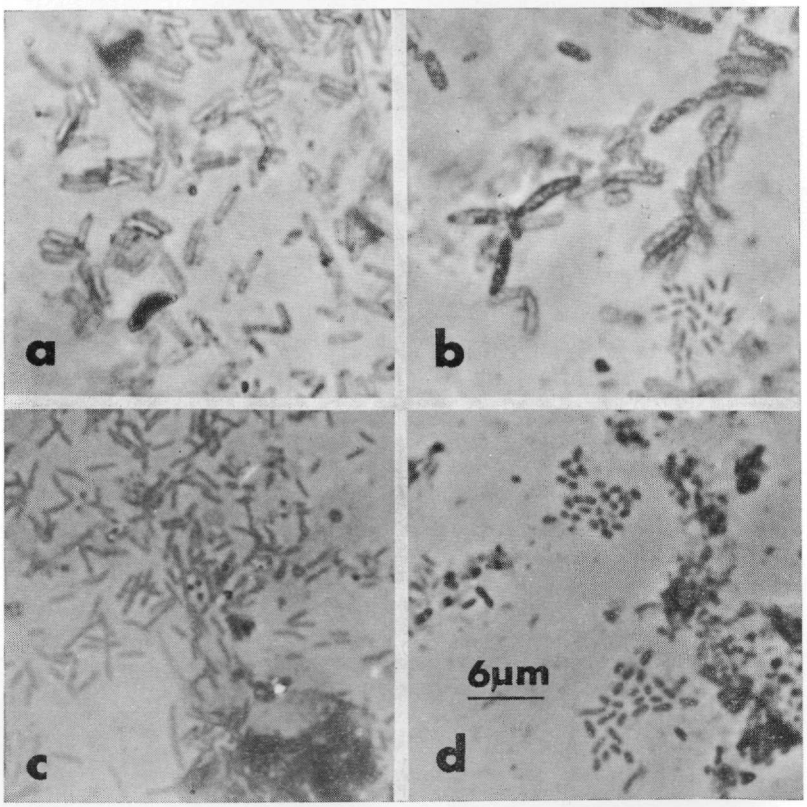

FIGURE 1. Bacteria and debris found at the water-air interface after 4 day (a), 6 day (b), and 7 day (c, d) incubation in mud jar on chitin coated slides. Rod shaped organisms are seen in (a) and (c) while granular rods (b) and ovoid cells (d) are also evident. Bar represents 6 μm in all photomicrographs.

TABLE 1.

*Gross changes in chitin coated buried slides with increased times.**

Zones	No Chitin	Chitin					
	7 DAY†	2 DAY	4 DAY	6 DAY	7 DAY	10 DAY	13-20 DAY
Water Surface	1. **	1. None	1. Slight	1. Nearly complete	1. Nearly complete	1. Complete	1. Complete
	2. Few	2. Numerous	2. Numerous	2. Numerous	2. Numerous	2. Decreasing numbers	2. Few
	3. Actinomycetes	3. Rods of 3 μm	3. Rods	3. Rods	3. Rods	3. Spores	3. Spores
Water	1. **	1. None	1. None	1. Nearly complete	1. Nearly complete	1. Complete	1. Complete
	2. Few	2. Numerous	2. Numerous	2. Numerous	2. Numerous	2. Decreasing numbers	2. Few
	3. Cocci and spores	3. Rods	3. Rods of 2 μm	3. Ovoid cells	3. Ovoid cells	3. Spores	3. Slightly spiral cells
Mud Surface	1. **	1. None	1. None	1. Slight	1. Slight	1. Nearly complete	1. Complete
	2. Few	2. Some	2. Some	2. Some	2. Numerous	2. Numerous	2. Numerous
	3. Actinomycetes	3. Rods and cocci	3. Rods and ovoid cells	3. Large rods and ovoid cells	3. Ovoid	3. Filaments and ovoids	3. Vibrioids and ovoids
Aerobic Mud (0–2 cm)	1. **	1. None	1. None	1. Slight	1. Slight	1. Slight	1. Partial
	2. Few	2. **	2. Few	2. Some	2. Some	2. Increasing numbers	2. Numerous
	3. Rods	3. **	3. Cocci and rods	3. Slender, slightly curved rods	3. Ovoid and slightly curved cells	3. Slightly curved cells and rods	3. Vibrioids
Anaerobic Mud (2–5 cm)	1. **	1. None	1. None	1. None	1. Slight	1. Slight	1. Slight
	2. **	2. **	2. **	2. **	2. Some	2. Increasing numbers	2. Numerous
	3. **	3. **	3. **	3. **	3. Large diversity: vibrioids, ovoids, rods	3. Vibriods and spirals	3. Long spirals and vibrioids

*1=comparison of degree of chitin hydrolysis, 2=bacterial number associated with slide, and 3=predominant bacterial types.

**Does not apply.

†Incubation time.

bation period. Table 1 indicates the colonization by vibrioid and filamentous forms (10 to 20 day). Figure 2b shows typical cells after a 4 day incubation period at the water-sediment interface. The types evident at this water-sediment interface and in the aerobic sediment are seen in fig. 2b through d. These morphologic types did not differ greatly from those found in the first 2 cm of sediment. Figure 2c shows rod shaped cells growing on a mat of chitin with some hydrolysis evident around certain microorganisms. Figure 2d shows a small mat of chitin being solubilized by vibrioid and rod shaped organisms after 17 day incubation. This finding points out a rather stable population in the sediment when considering morphologic types. It also points to the existence of microcosms slowly hydrolyzing chitin since most chitin was cleared from the slide at this time in the aerobic zone.

Anaerobic decomposition was the slowest process, as evidenced by only slight clearing of the chitin film on the slide (table 1). The distinctly different morphologic types may be seen in figure 3a through b. Active hydrolysis is noted by the clearing observed (see fig. 3a). The variety is readily seen in this photograph of a slide after a 10 day incubation period. Figure 3b shows an area of partial hydrolysis noted after 13 day incubation in the anaerobic sediments. Large spirilla and vibrioid cells are evident in both photographs. Figure 3c shows the typical spore-like cells (refractile bodies) found on the surface of the control (uncoated) slide during most of the time

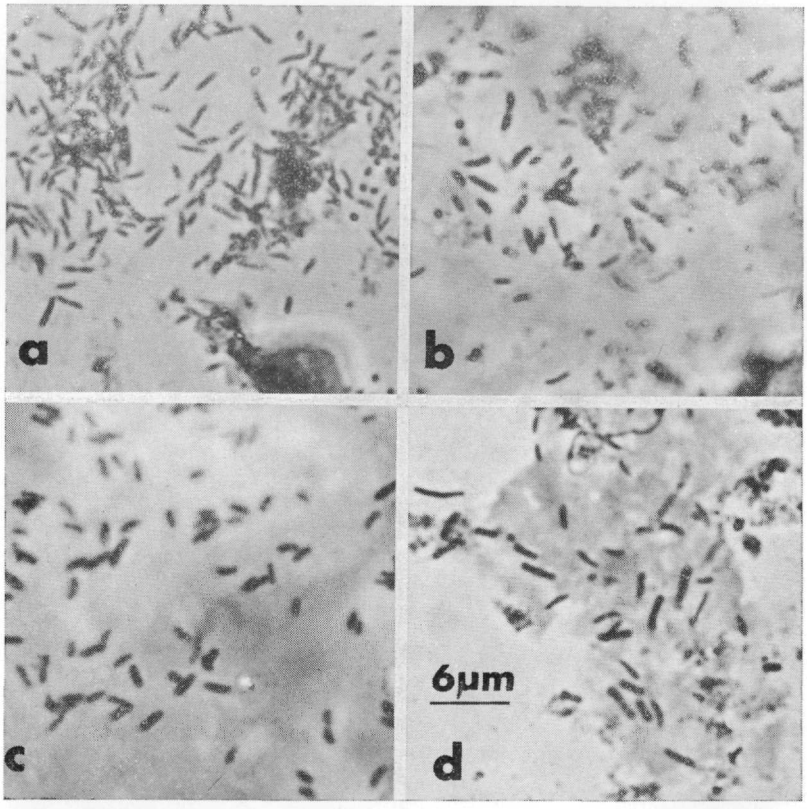

FIGURE 2. Bacteria and debris associated with chitin coated slides in the water column (a) and aerobic mud zones (b-d) are seen. Photomicrograph of water column was taken after 6 day incubation while photomicrograph of mud surface (b) was taken after 4 day incubation. Aerobic sediment photomicrographs were removed and examined after 4 (c) and 17 (d) days incubation. Bar represents 6 μm in all photomicrographs.

period regardless of location in the water or sediment regime.

DISCUSSION

Examination of the photomicrographs taken of the chitin coated slides led to a number of general conclusions that may be made concerning chitinolytic processes within the water column and sediments. Hydrolysis of chitin was more rapid within the water column and the aerobic sediments. These results substantiated work done by Gray and Baxby (1968) in the marine environment. The microorganisms responsible for this phenomenon appeared to be bacillary microorganisms. Using a similar technique as applied in our study, Okafor's (1966) findings pointed to a predominance of coccoid microorganisms within chitin amended soil. This finding was not evident in the present study. In all studies cited, the numbers of bacteria increased significantly when the environment had been enriched with colloidal chitin, as is the case with our study.

The anaerobic processes were slower for two probable reasons: the particulate

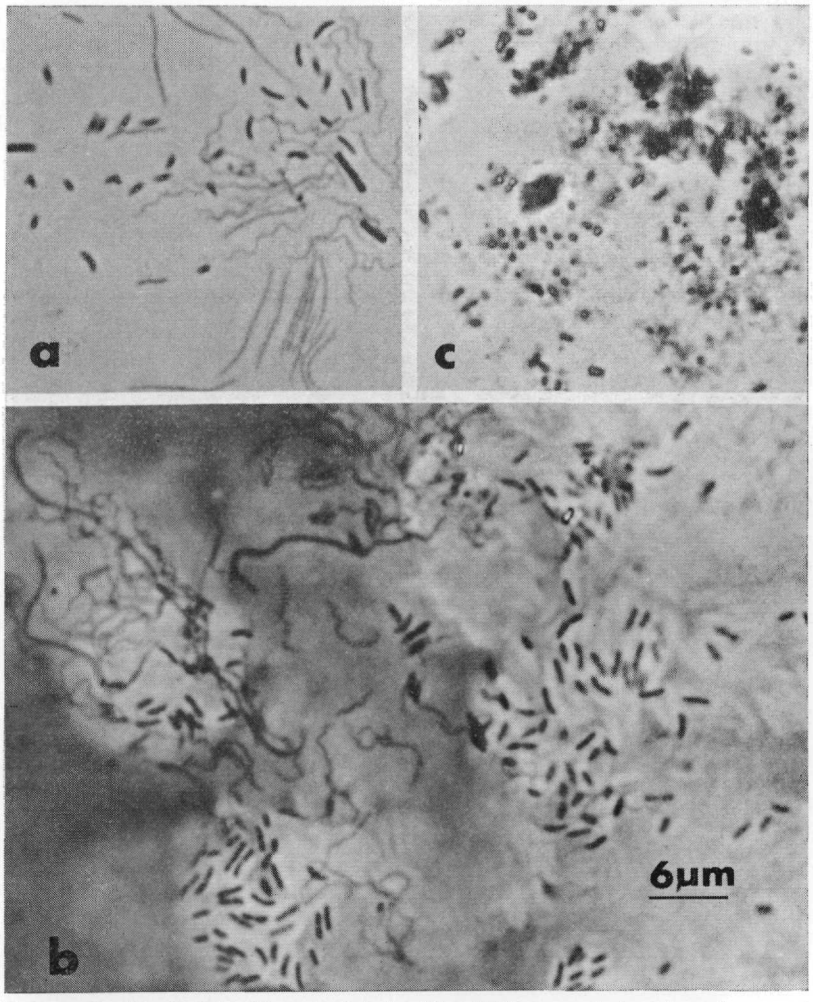

FIGURE 3. Bacterial cells associated with chitin coated (a, b) and control slides (c) after 10, 13, and 7 days of incubation, respectively. Vibrioid and spiral cells predominate in the anaerobic sediment (a, b) while refractile spore like cells were evident on the control slide (c) on all days examined. Bar represents 6 μm in all photomicrographs.

nature of the sediments and the lower rate of metabolism of anaerobes in general. Anaerobic degradation appeared to be initiated by rod-shaped organisms, but these appeared to lose ground to the vibrioid and spiral microorganisms as time progressed. Such types were commonly found in aquatic sediments by Benton (1935). Actinomycetes, normally considered to have chitinolytic properties, were not observed in large quantities by this adaptation of the buried slide procedure. This did not, however, exclude their activity within this environment but merely indicated the possible failure of the technique to allow the actinomycetes to effectively compete with the other organisms or their inability to attach to the slide.

Whether the activity evidenced on these slides by varying bacterial numbers and types is truly indicative of the processes occurring in nature is only speculative; however, Warnes and Randles (1977) and Warnes (1972) enumerated potentially chitinolytic bacteria in sediments and the water of Lake Erie, respectively. The former study showed greatest numbers at the sediment water interface with decreasing numbers occurring with increasing depth of the sediment. A leveling off was noted in the relatively stable anaerobic sediments, lending credence to the findings displayed on table 1.

The succession evidenced on the chitin coated slides likely occurs on many chitinous substrates in nature, such as an exoskeleton, decaying, fungal filaments, or other sources. As in this experiment, a major portion of the chitin is removed rapidly by aerobic processes leaving the remnants to settle into an anaerobic zone. When the substrate is subjected to anaerobic degradation, the process is slower due in part to the lowest rates of metabolism of anaerobic organisms. Since suspending slides *in situ* in water or sediment in a highly used body such as Lake Erie is impractical, this type of study yields some information as to processes involved in a mixed community not otherwise obtainable.

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